Over-Summering and Biotypic Diversity of *Schizaphis graminum* (Homoptera: Aphididae) Populations on Noncultivated Grass Hosts

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ABSTRACT Greenbug, *Schizaphis graminum* (Rondani), populations over-summering on noncultivated grass hosts may be implicated in early fall infestations in wheat. The purpose of this study was to examine the relationship between over-summering greenbugs on noncrop hosts and fall infestations on wheat. Since greenbug populations on noncultivated hosts may also act as reservoirs of virulence genes, the biotypes of collected aphids were also determined. The grass species present at three sites (two in Oklahoma and one in Kansas) were identified and a species richness curve was generated. Greenbugs were collected at these sites and their hosts and biotypes determined. At Hays, KS, a persistent over-summering greenbug population lead to an early fall infestation in wheat. At the sites in Oklahoma, where over-summering greenbugs were not detected, the fall infestation occurred 3 months later. Biotypes G, I, K, and a new biotype (i.e., previously undescribed) were found on noncultivated hosts at Hays, but only biotypes I and K were found on the cultivated wheat. Finding a new biotype supports the hypothesis that biotypic diversity (new combinations of virulence genes) is generated and maintained on noncultivated grasses, which may then act as reservoirs of virulence genes found in populations on crops.

KEY WORDS greenbug, biotypes, plant resistance genes, insect-plant interactions

THE GREENBUG, *Schizaphis graminum* (Rondani), feeds on a variety of graminaceous plants and is a serious pest of cultivated grasses (e.g., wheat, barley, and sorghum). Cultivated crop species represent a very small proportion of the 70 greenbug hosts representing 29 genera, (Michels 1986). Noncultivated hosts are an important component of greenbug population dynamics. Grass hosts are crucial for greenbug survival during the summer, particularly after the wheat harvest in areas in which sorghum is not grown. During this time, greenbugs are found on noncultivated grasses (Daniels 1961) and volunteer wheat. From 1953–1959 greenbugs were found over-summering on 23 grass species in the Texas panhandle (Daniels 1960).

Noncultivated grasses are important during the sexual cycle, which occurs, in broad terms, north of the 35th parallel in the United States. Generally, sexual morphs are produced and eggs laid before the emergence of fall planted wheat. Greenbugs oviposit on grasses, especially *Poa* spp. (Dixon and Kundu 1994). Because the fundatrix emerges onto these grasses in the spring, initial selection pressures are exerted by these noncultivated grasses. In contrast, greenbug populations on wheat become extinct before sexual morphs are produced, because wheat matures and is harvested long before the sexual cycle is initiated. Therefore, they rarely contribute to the next year's population.

Besides acting as temporal and spatial reservoirs, noncultivated grasses may also serve as reservoirs of genetic diversity for virulence and other characters. Some greenbug clones have been found to cause a virulent reaction in a plant when feeding. Greenbug clones have traditionally been assigned biotypic status on the basis of virulence to specific plant genotypes (Table 1.). The biotypic diversity (diversity of virulence genes) has traditionally been measured on crop hosts (Peters et al. 1997, Dumas and Mueller 1986), however, noncultivated hosts were ignored. There is evidence to suggest these virulence genes have evolved on noncultivated hosts. Porter et al. (1997) found little or no correspondence between the introduction of resistant wheat and sorghum cultivars and the emergence of virulent biotypes. Instead, they proposed that greenbug populations may be a complex of host-adapted races that evolved on noncultivated hosts along with associated virulence and avirulence genes.

Mitochondrial DNA sequences showed that greenbug populations were divided into three infraspecific clades (Shufran et al. 2000). Biotypes collected regularly from crop hosts were confined to one clade,

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Table 1. Plant reactions (R = resistant, S = susceptible) to known greenbug biotypes[a] and greenbug isolates collected from Canada wild rye (CWR[b]) (*Elymus canadensis*) and western wheatgrass (WWG) (*Agropyron smithii*)

Plant entry	Gene designation	Greenbug biotype											
		A	B	C	E	F	G	H	I	J	K	CWR[b]	WWG
Wheat													
Custer		S	S	S	S	S	S	S	S	R	S	S	S
DS 28A	*Gb1*	R	S	S	S	R	S	S	S	R	S	S	S
Amigo	*Gb2*		R	R	S	S	S	S	S	R	S	R	S
Largo	*Gb3*		S	R	R	S	S	R	R	R	R	S	S
CI17959	*Gb4*		S	R	R	S	S	S	R	R	R	S	R
CI17882	*Gb5*		S	R	R	S	S	S	R	R	R	S	R
GRS1201	*Gb6*		R	R	R	S	R	S	R	R	R	S	R
Rye													
Elbon			S	S	S	S	S	S	S	R	S	S	S
Insave	*Gb2*, *Gb6*		R	R	R	S	R	S	R	R	R	R	R
Barley													
Wintermalt			S	S	S	S	R	S	S	R	S	S	S
Post90	*Rsgla*		R	R	R	R	R	S	R	R	R	R	R
PI426756	*Rsg2b*		R	R	R	R	R	S	R	R	R	R	R

[a] Data from Woods (1961), Porter et al. (1982), Kindler and Spomer (1986), Puterka et al. (1988), Harvey et al. (1991), Beregovoy and Peters (1994), and Harvey et al. (1997).
[b] For a description, see Shufran et al. (2000).

whereas the other clades contained biotypes rarely collected on crop hosts or collected from noncultivated hosts (Shufran et al. 2000). The authors postulated that these clades represented host-adapted races that evolved on noncultivated grasses. This hypothesis was supported by Anstead et al. (2002) who showed a correspondence between clade and host use. Gene flow between races would allow new combinations of virulence genes (biotypes) to emerge and potentially affect crops. The objective of the current study was to determine the biotypic diversity of greenbug populations on noncultivated hosts. We also assessed the role of these populations in causing early autumn infestations in wheat.

Materials and Methods

Sampling. Greenbug collections were made from three sites; Hays, KS (N 38° 50′ 32″, W 99° 18′ 09″), Redrock, OK (N 36° 26′ 34″, W 97° 06′ 84″), and Marshall, OK (N 36° 06′ 53″, W 97° 36′ 08″). The areas were chosen for their diversity of noncultivated grasses and because each site bordered a cultivated wheat field. During the study, the grasses were not disturbed (i.e., mowed, grazed, or burned). Each study area was 40 m × 10 m.

In June 1999, the grasses at each site were identified to species. The 40-m long fence line between the noncultivated and cultivated areas served as the sampling transect. Starting 10 m from the end of the fence line, at 1-m intervals, a 10-m transect perpendicular to the fence line was sampled. Each grass touching the line was identified by James Anstead using Hitchcock and Chase (1971). Cool season annuals were identified from the previous season's culms and inflorescences, and were included in the curve. The cumulative number of grass species (species richness) found was plotted against the cumulative distance examined. If a grass could not be identified in its vegetative state, it was either marked and determined at a later growth stage, or a small amount was removed to the laboratory for identification. Sampling was terminated after five 10-m transects had been sampled because the species richness curves indicated that all grass species should have been detected (Southwood 1978). During subsequent sampling for greenbugs at each site, more than 1,000 grass culms were examined and determined to species. All species initially identified were later found again, however, only one additional *Aegilops cylindrica* host plant was found at Hays.

Aphids were collected between May 1999 and March 2000 at approximately 1 mo intervals. Sampling was conducted by randomly casting a 0.25-m^2 quadrat into the plot. A quadrat 0.5 m away from the cast was then sampled. Twenty culms of each grass species in the quadrat were examined manually for aphids. If less than 20 culms were present, then all culms were examined. On each sampling date, 20 quadrats were examined. To determine the presence and absence of greenbugs in the adjacent wheat field, 20 quadrats were also checked. At Hays, greenbugs were collected from volunteer wheat in August and from cultivated wheat in September and October, and their biotype determined. Greenbugs were collected by cutting out and removing a small section of the grass hosting the colony. This grass fragment was transferred with the colony still attached to a small glass vial and moved to the laboratory within 24 h.

In the laboratory, single greenbugs were used to start clonal colonies by transferring to an "Otis" barley plant using a paintbrush. Each clone was maintained on Otis barley grown in a 3.65-cm × 21-cm Conetainer (Cone-tainer Company, Canby, OR) covered with a cylindrical plastic cage. The cages are manufactured from plastic tubing, with several cloth covered ventilation holes in the sides. Barley plants were grown in fritted clay (near-sterile media) and watered and fertilized by placing the Conetainer racks in a tray of water containing Peters Professional 20-20-20 fer-

tilizer at 1 teaspoon per gallon. After the aphids were caged on the barley, watering was discontinued to reduce humidity inside the cage and thus reduce fungal infection. The plants were then kept in a growth chamber at 17 ± 3°C with a photoperiod of 14:10 (L:D) hours. Because greenbugs quickly kill barley, each clone was transferred to a new plant approximately every 2 wk.

Insect vouchers were deposited at the Cereal Insect Genetic Resource Library, USDA–ARS, Plant Science and Water Conservation Research Laboratory, Stillwater, OK. James Anstead holds grass vouchers.

Determination of Biotypes A, B, C, F, G, H, and J. The biotype of each greenbug clone was determined using previously established plant differentials of wheat, barley, and rye (Table 1). The aphids were allowed to reproduce on seedling barley in four rectangular pots (15 cm × 8 cm). A single host was used for increasing rearing greenbugs for testing purposes. Previous studies showed no differences in maternal effects when raised on different hosts and subsequently transferred to sorghum, wheat, and barley (Beregovoy et al. 1988). Approximately 35–40 heavily infested plants, yielding 400–800 aphids, were produced for each test. For each test, three sets of three plants for each differential were randomized in a 30-cm × 30-cm flat of polystyrene wells (5 cm × 5 cm × 5 cm) containing fritted clay. The plant entries used were found to have varying germination times, therefore planting dates were staggered to ensure all the plants were at the two-leaf stage before infestation. "Wintermalt" and "Post 90" barley, "Insave" rye, and GRS1201 wheat were planted first. Twenty-four hours later, DS28A, "Amigo," CI 17959, and CI 17882 wheat were planted and, finally, after another 24 h, "Largo" wheat, PI 264453 rye, and "Custer" wheat were planted. To ensure efficient transfer, the aphids were introduced into the cage containing the plant differentials at the two-leaf stage by cutting and placing the infested barley leaves next to the test plants. The tests were maintained under artificial light (incandescent and fluorescent) with a photoperiod of 14:10 (L:D) hours, at 20 ± 5°C. After the susceptible control, Wintermalt barley, was killed (usually about 7 d), the test was terminated and the remaining plants were scored as alive or dead. If eight or nine of the plants were dead, then the differential was scored as susceptible. If none were dead, it was scored as resistant. If any entry didn't meet these criteria, it was retested until a determination could be made.

Determination of Biotypes E, I, and K. Testing with "Shallu," PI 264453, and PI 550610 sorghum was necessary to separate biotypes E, I, and K. "Shallu" is susceptible to biotypes E, I, and K. PI 264453 is resistant to biotype E, but susceptible to biotypes I and K. PI 550610 is resistant to biotypes E and I, but susceptible to biotype K. Therefore, if an aphid damaged only "Shallu," it was determined to be biotype E. If it damaged "Shallu" and PI 550610, it was determined to be biotype I, and if it damaged all three entries, it was determined to be biotype K (Harvey et al. 1997). For each test, three replicates of three seedlings were grown in Cone-tainers, in fritted clay, and covered with cages. The aphids were allowed to reproduce on seedling barley in four rectangular pots (15 cm × 8 cm). When the sorghum seedlings reached the two-leaf stage they were infested with equal numbers of aphids (between 15 and 20 in each test). The aphids were placed onto the plant with a camel-hair paintbrush to reduce transfer mortality.

After 14 d, the sorghum plants were visually rated for damage. If >60% of the plant was chlorotic or dead it was considered susceptible. This would correspond to a score of ≥7 on the conventional 1–9 scale of damage (1 = 0–10%, 9 = 81–100%) (Harvey et al. 1997). If fewer than 30% was damaged (a score of ≤4 on the old scale) it was considered resistant. Intermediate scores were retested. This new protocol was a simplification of the conventional 1–9 (1 = no damage, 9 = plant death) damage rating previously used to determine susceptibility to greenbug feeding.

Table 2. Summary of hosts sampled and greenbugs found at three sites

Site	Host	Number of culms sampled	Number of culms with greenbug
Hays, KS	*Aegilops cylindrica*	1	0
	Agropyron smithii	977	7
	Bouteloua curtipendula	2	0
	Bromus tectorum	410	13
	Cynodon dactylon	128	0
	Elymus virginicus	213	0
	Poa pratensis	166	0
	Setaria viridis	104	1
	Sporobolus sp.	8	0
Redrock, OK	*Bromus catharticus*	254	0
	Bromus tectorum	297	0
	Cynodon dactylon	43	0
	Elymus canadensis	78	0
	Eragrostis sp.	456	0
	Hordeum pusillum	4	0
	Panicum virgatum	520	0
	Phalaris canariensis	33	0
	Sorghum halepense	22	1
	Tridens flavus	54	0
Marshall, OK	*Aristida oligantha*	23	0
	Bothriochloa saccarhoides	243	0
	Bouteloua curtipendula	7	0
	Bromus catharticus	312	0
	Chloris virgata	421	0
	Cynodon dactylon	45	0
	Echinochloa crusgalli	233	0
	Elymus canadensis	67	0
	Eragrostis spectabilis	23	0
	Scheddonardus paniculatis	12	0
	Setaria viridis	3	0
	Sorghum halepense	43	3

Results

There were 11 grass species present at Marshall, OK, 10 at Redrock, OK and nine at Hays, KS (Table 2). *Cynodon dactylon* (L.) was the only species common to all three sites. The collection data are summarized in Fig. 1, and include whether greenbugs were present in the wheat field bordering the study plot. At Hays, the neighboring field contained considerable volun-

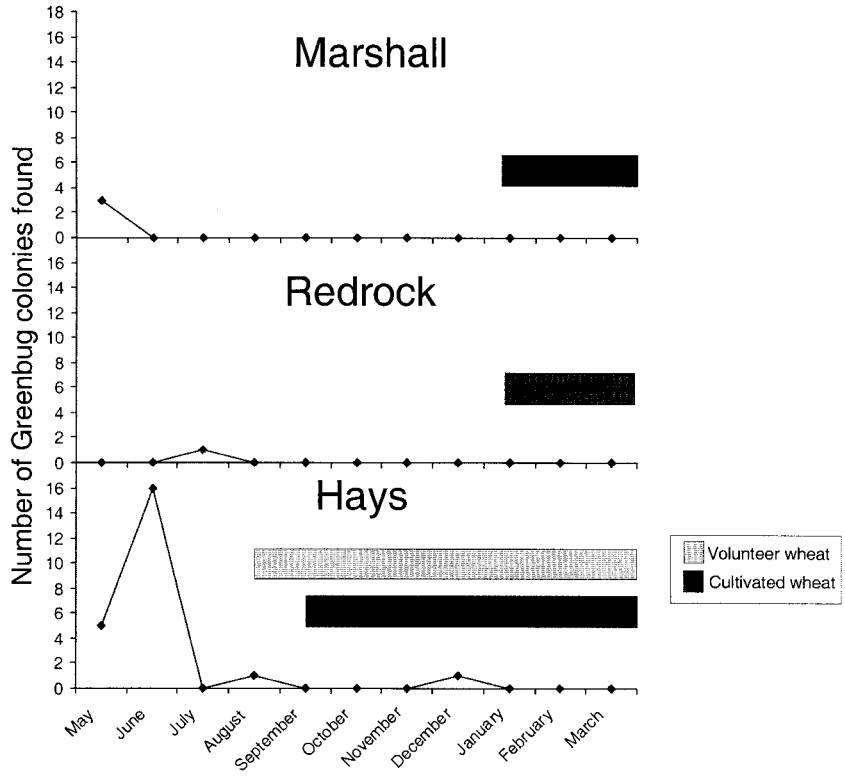

Fig. 1. The temporal distribution of greenbug colonies in noncultivated grasses at three sites during 1999–2000, including the presence of greenbugs on adjacent volunteer and cultivated wheat.

teer wheat and presence/absence of greenbugs was recorded. Greenbugs were present in Hays throughout the study period, initially on noncultivated grasses and later on volunteer and cultivated wheat. At the other sites they were only found once on noncultivated hosts, but had become established on cultivated wheat by November.

Greenbug densities were low on noncultivated hosts even at Hays (Table 2). For example, of 977 culms of *Agropyron smithii* Rydberg examined, only nine had at least one greenbug. Many findings consisted of a single aphid. At Hays, greenbugs were found on *A. smithii*, *Bromus tectorum* L., and *Setaria viridis* (L.), but not on any of the other grasses, which accounted for 25% of the ≈2,000 grass culms examined.

On noncultivated hosts, biotype I was the predominant biotype. It was present on every host (Table 3) and accounted for 61% of the greenbugs collected. Biotype E was second most common (18%), followed by biotype G (15%) and biotype K (6%). On wheat, biotype I (71%) also was predominant, followed by biotype E (17%) and biotype K (12%). It should be noted that all biotype G isolates also showed a biotype I virulence profile on sorghum. Therefore, biotype testing with sorghum entries alone (i.e., excluding the wheat, barley, and rye entries) would have lead to an incorrect determination of these isolates and an overestimation of the frequency of biotype I. A "new" biotype was also found based on its virulence to the *Gb3* resistance gene (Tyler et al. 1987). It was collected from *Agropyron smithii* (western wheatgrass) in Hays, KS, in June 1999. It showed the same virulence profile as biotype I except it was also virulent against Largo wheat. We refer to it as the western wheatgrass (WWG) isolate.

On wheat, every aphid clone expressed virulence to DS28A and Amigo (containing plant resistance genes *Gb1* and *Gb2*, respectively). Virulence to CI 17959 (*Gb4*) and CI 17882 (*Gb5*) is expressed in biotype G and was therefore present in 15% of the isolates. Virulence to Largo (*Gb3*) is expressed in biotype G isolates and in the WWG isolate, therefore it was present in 18% of the samples. On sorghum, all greenbugs collected expressed virulence to Shallu, 82% expressed

Table 3. Summary of greenbug biotypes collected from noncultivated grasses, volunteer wheat, and cultivated wheat

Grass Host	Number of colonies	Number biotypes	Biotypes identified
Agropyron smithii	7	4	I, G, K, WWG*
Bromus tectorum	13	2	I, G
Sorghum halepense	4	2	I, E
Setaria viridis	1	1	I
Volunteer wheat	20	3	E, I, K
Cultivated wheat	25	3	E, I, K

* Western wheatgrass isolate.

virulence to PI 264453, and 6% expressed virulence to PI 550610.

Discussion

At all sites, greenbug densities were very low. The Redrock, OK, and Marshal, OK, sites showed a very similar pattern of greenbug presence in the wheat and grasses (Fig. 1). In both sites, greenbugs were only detected once in the noncultivated grasses. However, a fall infestation was found in the adjacent wheat and persisted into the spring. It seems likely these infestations were the result of regional rather than local movement, especially considering that without noncultivated hosts these populations cannot persist once the crop has matured. Greenbugs present on wheat may have lacked the ability to use nearby grasses, even though the same grass species supported greenbugs at other locations. It is possible they didn't colonize these grasses because of (1) poor host quality, (2) they were not adapted to these particular hosts, or (3) the efficiency of natural enemies was higher in the noncultivated areas. Greenbugs using crop hosts have been shown to possess low genetic diversity and to be divergent from populations found on noncultivated hosts (Anstead et al. 2002). These greenbugs may have been maladapted to grasses other than wheat.

At Hays, KS, greenbug populations that over-summered on noncultivated grasses recolonized the wheat in autumn. Moreover, greenbugs were present in cultivated wheat 2 mo earlier than in Marshall and Redrock. This is likely because of local movement of the over-summering greenbugs found at Hays, versus a later and more regional greenbug movement at the Oklahoma sites. At Hays, greenbugs were found on only three of the nine grass species sampled (Table 3), despite having all been listed as greenbug hosts (Michels 1986). This suggests the local population was only adapted to a subset of the grasses present. This supports earlier work that show individual greenbug clones differed in their ability to survive on different grass species (Dahms et al. 1954, Kindler and Hays 1999, Anstead 2000). Host specialization has also been found in other aphid species. Particular clones of *Sitobion avenae* F. preferred to colonize and performed better on wheat than other grasses (De Barro et al. 1995). Similarly, *S. avenae* lineages separated by a single-locus microsattelite and a mitochondrial marker were found to exhibit host specialization (Sunnucks et al. 1997a). Three host-adapted races were also found within *Therioaphis trifolii* (Monell) populations (Sunnucks et al. 1997b).

Biotype I populations were composed of a number of clones that were collectively able to use a wide variety of hosts. It was present on all hosts that were able to support greenbug populations (Table 3). This conclusion is supported by Anstead et al. (2002), who identified three clades within the greenbug based on a mitochondrial gene tree. These clades may represent host-adapted races. Because biotype I contained clones from each of these clades, biotype I would be expected to have a large host range.

Finding biotype G at such high densities (15% of clones tested) was unexpected. In previous surveys it accounted for a maximum of only 2–3% of greenbugs collected from crop hosts (Bush et al. 1987, Ullah 1993). Furthermore, we found biotype G almost exclusively on *Agropyron* spp. and not from volunteer and cultivated wheat. It appears biotype G is adapted to a limited set of noncultivated grass hosts, however, further collections and host range studies are needed to confirm this.

Recombination of virulence and avirulence genes during meiosis can lead to the formation of "new biotypes" (Puterka and Peters 1990). Hays, KS is north of the 35th parallel, which is the approximate demarcation line at which sexual reproduction begins to occur (Wadley 1931). Therefore, the discovery of a "new" biotype was not totally unexpected. The WWG isolate was likely the result of sexual reproduction at this site. The WWG isolate and biotype I both expressed virulence to DS28A and Amigo (*Gb1* and *Gb2*), but the WWG isolate also expressed virulence to Largo (*Gb3*). Because both biotypes G and I (virulent to Largo) were present at Hays, matings between these two biotypes were likely to occur; the progeny of which might then elicit the WWG biotype feeding reaction. However, this would need to be verified in the laboratory by reciprocal crosses. This demonstrates how virulence genes present in populations on noncultivated hosts may form new combinations that could eventually threaten resistant crops. The discovery of the WWG isolate is similar to that of the CWR isolate, which was also found on a wild grass in Stillwater, OK (Shufran et al. 2000). Stillwater is located at N 36°, an area in which greenbug populations express the sexual life cycle.

In summary, the potential of noncultivated hosts to provide reservoirs of greenbugs that may cause earlier fall infestation was demonstrated. Redrock and Marshall contained nonpersistent greenbug populations that had immigrated from elsewhere and were not adapted to the grasses present locally. Hays, however, contained persistent greenbug populations able to use wheat and noncultivated grasses. Biotype I was found on a wide variety of hosts, whereas biotype G was shown to have a much more limited host range. The discovery of another greenbug isolate (WWG) from a wild grass that exhibits a new, previously undescribed virulence profile to plant resistance genes, further argues the importance of the role noncultivated hosts play in the evolution and maintenance of greenbug biotypes.

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